Microenvironnement et angiogenèse des métastases hépatiques :

Raphaëlle Audollent, Philippe Bonnin, Dong Broqueres-You, Jean-Philippe Brouland, Clarisse Eveno, Evelyne Dupuy, Chetana Lim, Marc Pocard

Unité 965 INSERM : Carcinose angiogenèse et recherche translationnelle

Hôpital Lariboisière Paris

Marc POCARD

Inserm

Institut national
de la santé et de la recherche médicale

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

université PARIS DIDEROT

RENAPE
CENTRE NATIONAL DE RÉFÉRENCE DES TUMEURS RARES DU PÉRITOINE

Liens d'intérêts 2012-2016

Honoraires / Consultant : GAMIDA

LEO Pharm; IPSEN; ROCHE; SANOFI

•Financement direct projets – congrès :

Plasma Jet; ETHICON; FUJINON;

GAMIDA; GENENTECH; IPSEN;

INTUITIVE surgical; LEO Pharm;

RAND; ROCHE; STORZ; SANOFI

Back to history

- **Seed and soil theory**

Only used during many decades to focus on specific organ to detect early metastatic locations, as liver imaging for colon cancer.

- **Targeting cancer cells**

TNM classification

Chemotherapy

Bruin *et al. BMC Cancer* 2010, **10**:662
http://www.biomedcentral.com/1471-2407/10/662

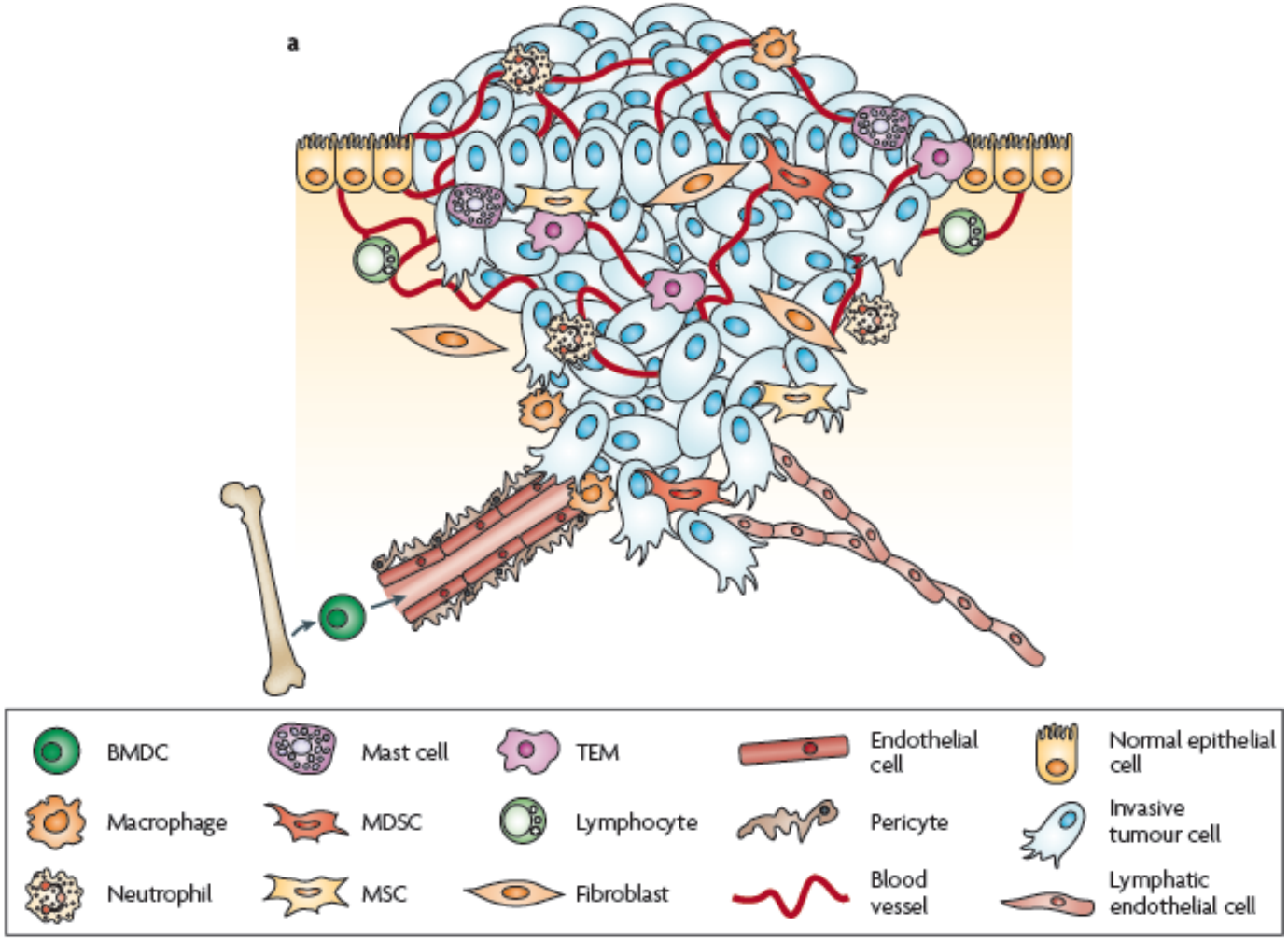
BMC Cancer

RESEARCH ARTICLE Open Access

Specific genomic aberrations in primary colorectal cancer are associated with liver metastases

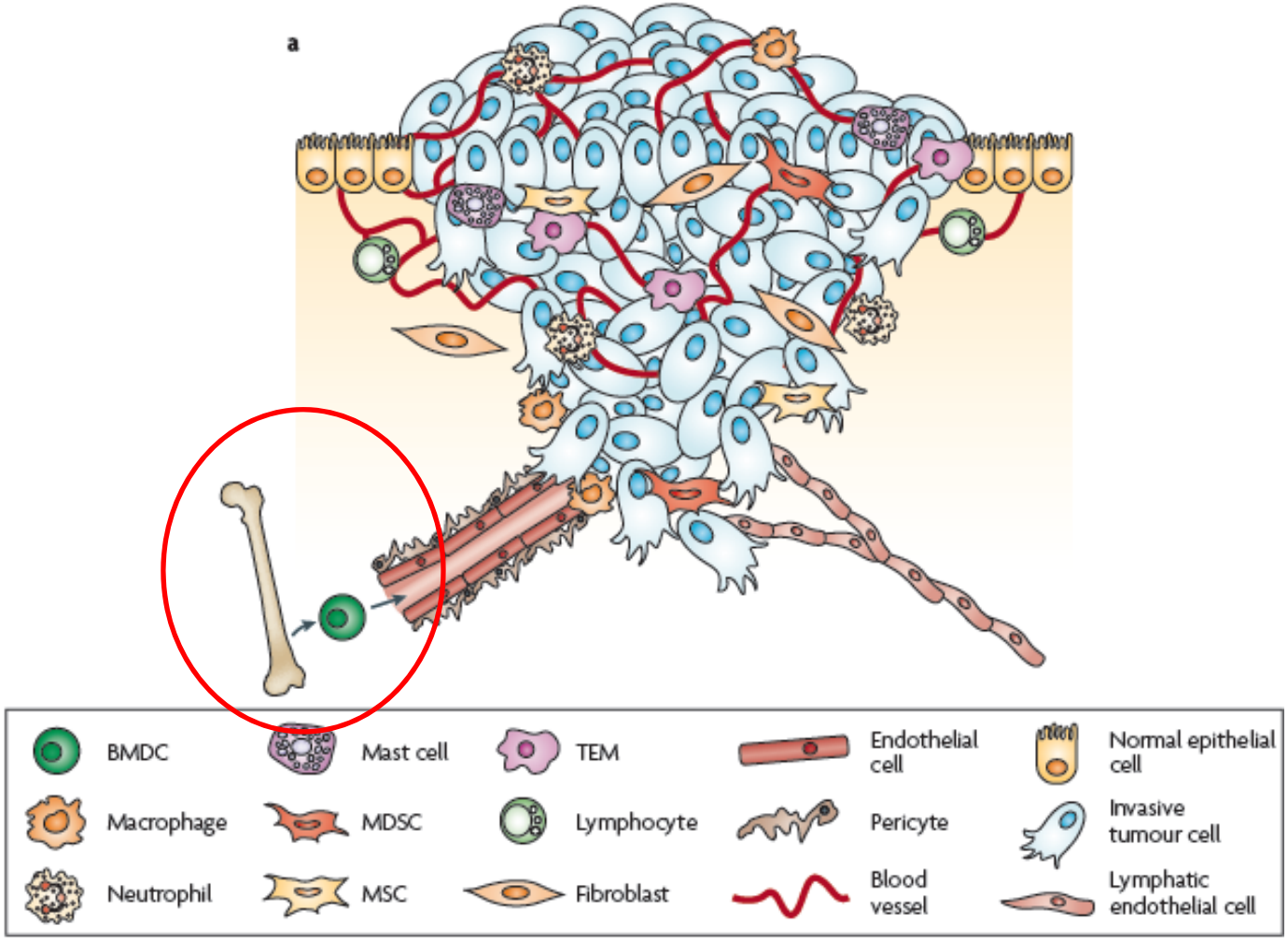
Sjoerd C Bruin[1], Christiaan Klijn[2,3], Gerrit-Jan Liefers[4], Linde M Braaf[4], Simon A Joosse[5,6], Eric H van Beers[5], Victor J Verwaal[7], Hans Morreau[8], Lodewyk F Wessels[2,3], Marie-Louise F van Velthuysen[9], Rob AEM Tollenaar[4], Laura J van't Veer[10*]

The tumor microenvironment



Joyce and Pollard. Nature Review Cancer. 2009

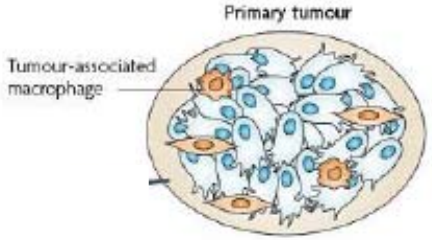
The tumor microenvironment



Joyce and Pollard. Nature Review Cancer. 2009

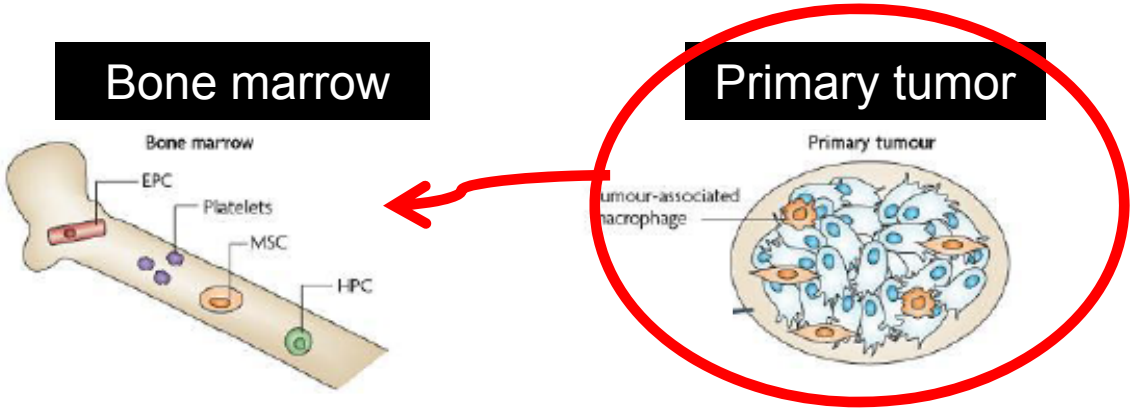
The pre-metastatic niche

Primary tumor



Peinado et al. Seminars in cancer biology, 2011
Psaila et al. Nature Review Cancer, 2009
Kaplan et al. Nature, 2005

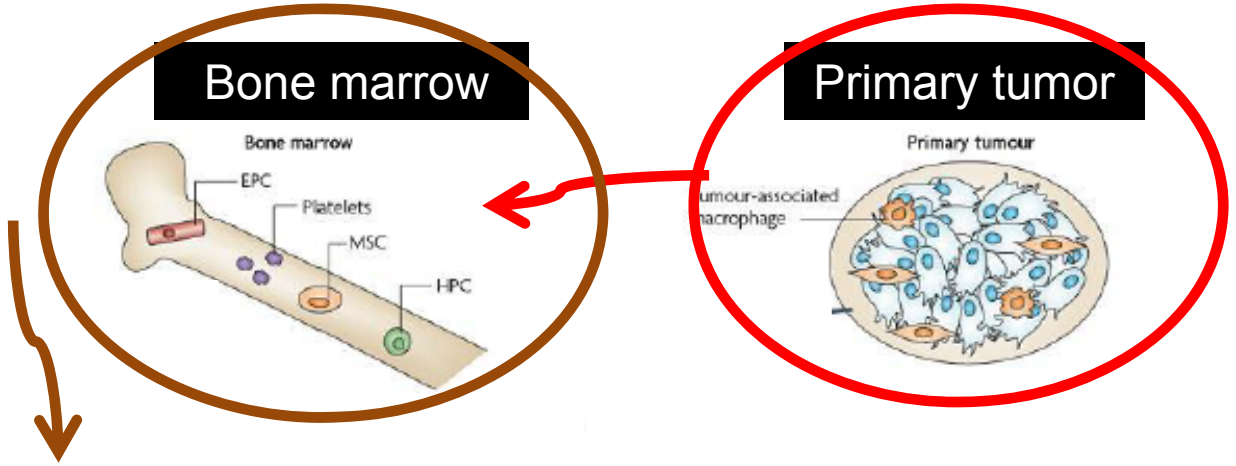
The pre-metastatic niche



Factors involved in mobilization of bone marrow stem cells

Peinado et al. Seminars in cancer biology, 2011
Psaila et al. Nature Review Cancer, 2009
Kaplan et al. Nature, 2005

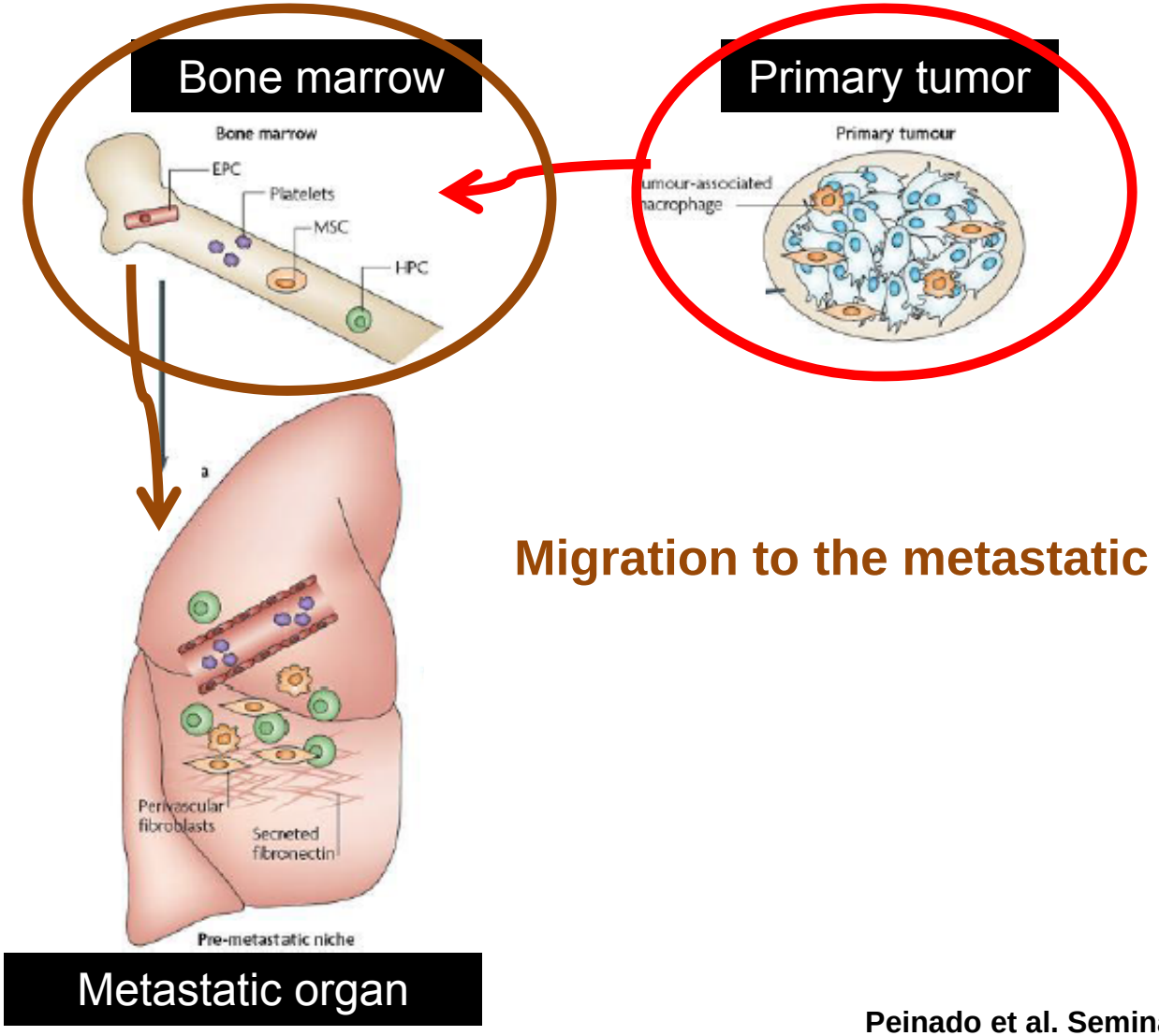
The pre-metastatic niche



Bloodstream

Peinado et al. Seminars in cancer biology, 2011
Psaila et al. Nature Review Cancer, 2009
Kaplan et al. Nature, 2005

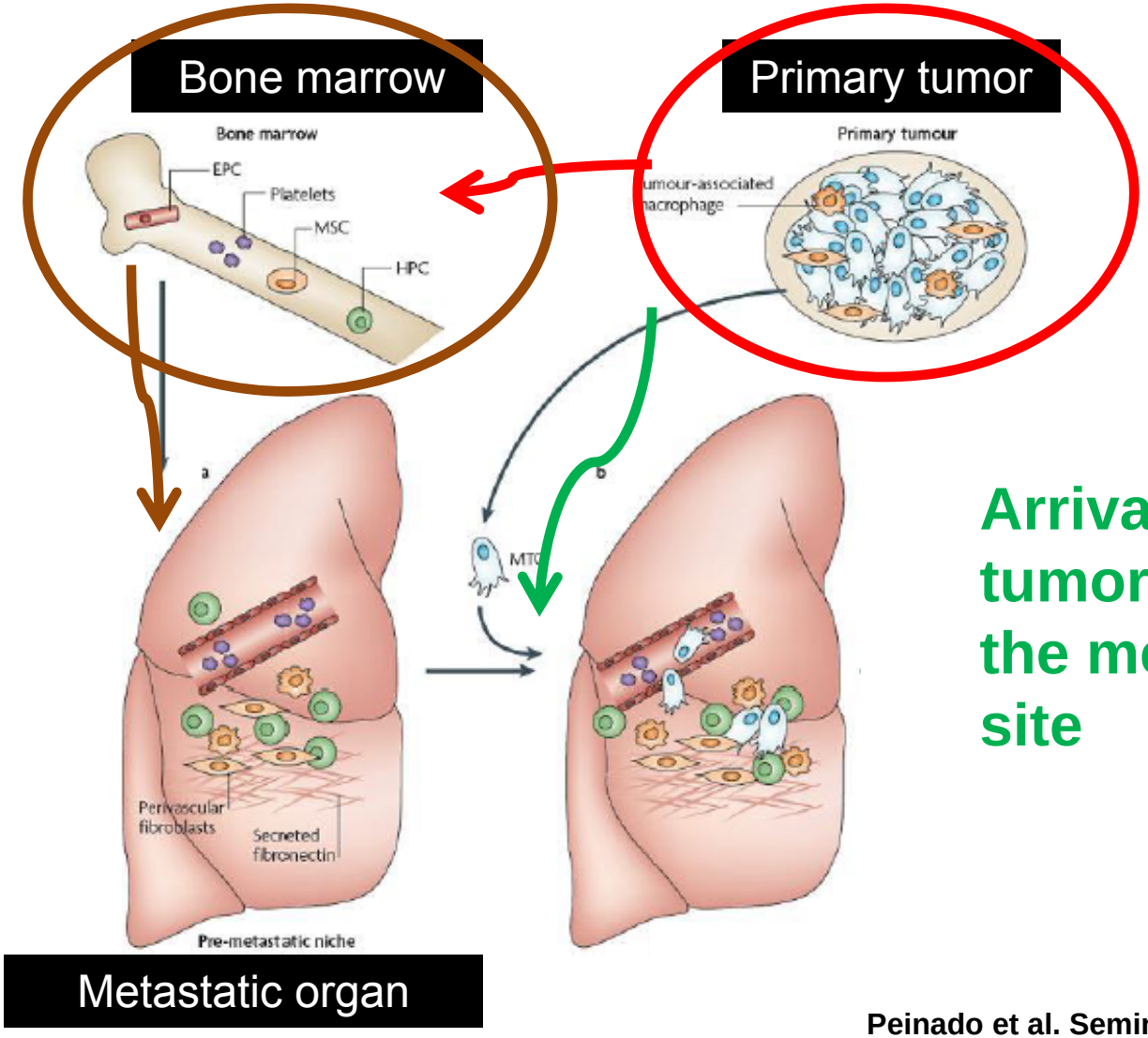
The pre-metastatic niche



Migration to the metastatic site

Peinado et al. Seminars in cancer biology, 2011
Psaila et al. Nature Review Cancer, 2009
Kaplan et al. Nature, 2005

The pre-metastatic niche



Arrivals of tumor cells in the metastatic site

Peinado et al. Seminars in cancer biology, 2011
Psaila et al. Nature Review Cancer, 2009
Kaplan et al. Nature, 2005

Bone-marrow derived cells

Impaired recruitment of bone-marrow–derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth

David Lyden[1,2], Koichi Hattori[2,5], Sergio Dias[5], Carla Costa[5], Pamela Blaikie[4], Linda Butros[1], Amy Chadburn[7], Beate Heissig[5], Willy Marks[3], Larry Witte[9], Yan Wu[9], Daniel Hicklin[9], Zhenping Zhu[9], Neil R. Hackett[6], Ronald G. Crystal[6], Malcolm A.S. Moore[2], Katherine A. Hajjar[8], Katia Manova[3], Robert Benezra[2] & Shahin Rafii[5]

nature medicine

NATURE MEDICINE • VOLUME 7 • NUMBER 11 • NOVEMBER 2001

VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche

Rosandra N. Kaplan[1,2,6*], Rebecca D. Riba[1,2*], Stergios Zacharoulis[1,2,6*], Anna H. Bramley[1,2], Loïc Vincent[4], Carla Costa[1,2], Daniel D. MacDonald[1,2], David K. Jin[4], Koji Shido[4], Scott A. Kerns[1,2], Zhenping Zhu[8], Daniel Hicklin[8], Yan Wu[8], Jeffrey L. Port[5], Nasser Altorki[5], Elisa R. Port[7], Davide Ruggero[9], Sergey V. Shmelkov[1,2,4], Kristian K. Jensen[1,2], Shahin Rafii[3,4] & David Lyden[1,2,6]

nature International weekly journal of science

NATURE|Vol 438|8 December 2005

Hypothesis

Modulation of the microenvironment of the cancer cells – immunodeficient animals

Promotion of tumor growth and metastasis

Animal orthotopic model

UICC
global cancer control

IJC
International Journal of Cancer

Bone marrow-derived endothelial and hematopoietic precursors cells enhance the metastasis of colon cancer in an orthotopic murine model

Raphaelle Audollent[1], Clarisse Eveno[1,2,3], Jean-Olivier Contreres[1], Patricia Hainaud[1], Aurore Rampanou[1], Evelyne Dupuy[1,3], Jean-Philippe Brouland[1,3,4] and Marc Pocard[1,2,3]

Human colon cancer cell lines LS174 : liver metastasis
Bone marrow derived cells (green florescent mice)
Co injected (tail vein) :
Result at day 38 Liver metastasis :
20% only cancer cells – 56% with bone marrow derived cell

uicc
global cancer control

IJC
International Journal of Cancer

Bone marrow-derived endothelial and hematopoietic precursors cells enhance the metastasis of colon cancer in an orthotopic murine model

Raphaelle Audollent[1], Clarisse Eveno[1,2,3], Jean-Olivier Contreres[1], Patricia Hainaud[1], Aurore Rampanou[1], Evelyne Dupuy[1,3], Jean-Philippe Brouland[1,3,4] and Marc Pocard[1,2,3]

Metastasis

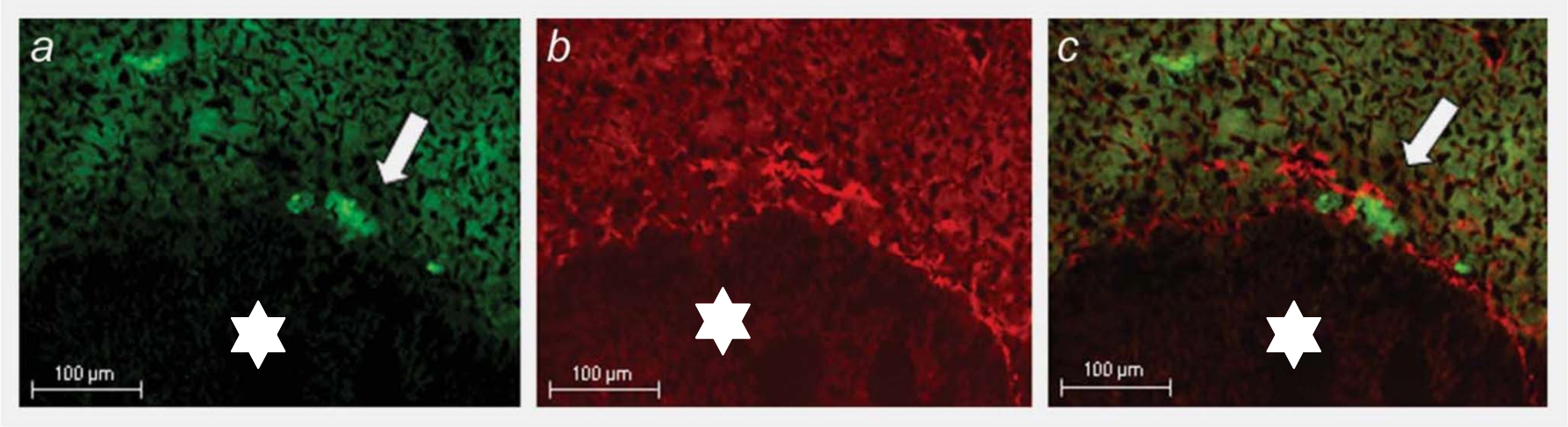


Figure 1. Migration of BMDCs to the stroma of a metastatic liver tumor (*): GFP-labeled BMDCs are localized to the tumor invasion front (*a*) and colocalized with endothelial cells (CD31 labeling in red) (*b*). (*c*) Merge (green and red).

Immuno- fluorescent staining of cut liver specimens has demonstrated that BMDCs labelled with Green fluorescent protein (GFP) always localized to the tumour invasion front and colocalized with endothelial cells

Hypothesis – immuno-competent animals

Modulation of the microenvironment of the cancer cells by bone marrow-derived cells

Promotion of tumor growth and metastasis

Rationale of our study

Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization

TOMONO TAKAHASHI, CHRISTOPH KALKA, HARUCHIKA MASUDA, DONGHUI CHEN, MARCY SILVER, MARIANNE KEARNEY, MEREDITH MAGNER, JEFFREY M. ISNER & TAKAYUKI ASAHARA

nature medicine

NATURE MEDICINE • VOLUME 5 • NUMBER 4 • APRIL 1999

Ephrin-B2–Activated Peripheral Blood Mononuclear Cells From Diabetic Patients Restore Diabetes-Induced Impairment of Postischemic Neovascularization

Dong Broquères-You,[1,2] Carole Leré-Déan,[1,3] Tatiana Merkulova-Rainon,[1,3] Chris S. Mantsounga,[1,3] David Allanic,[1,3] Patricia Hainaud,[1,3] Jean-Olivier Contrères,[1,3] Yu Wang,[2] José Vilar,[2] Marie Virally,[4] Jean-Jacques Mourad,[5] Pierre-Jean Guillausseau,[4] Jean-Sébastien Silvestre,[2] and Bernard I. Lévy[1,2,3]

American Diabetes Association. diabetes

Diabetes Publish Ahead of Print, published online May 17, 2012

Mice models

- **Liver metastasis of colon cancer**

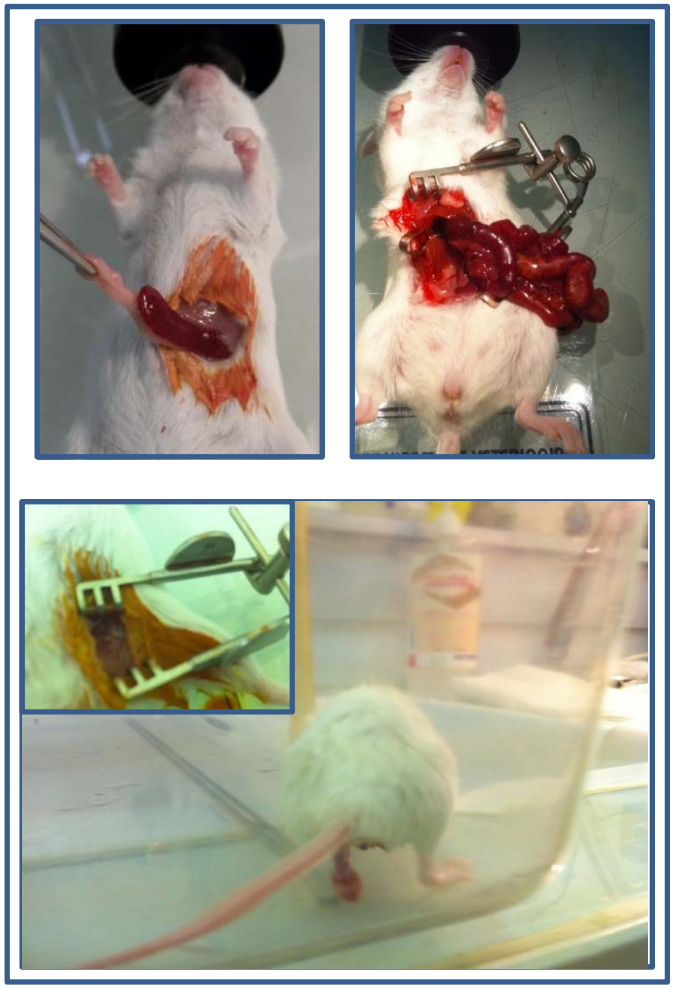
  (Intrasplenic injection of 1 million of CT-26 mouse cancer cells)

- **Unilateral hindlimb ischemia**

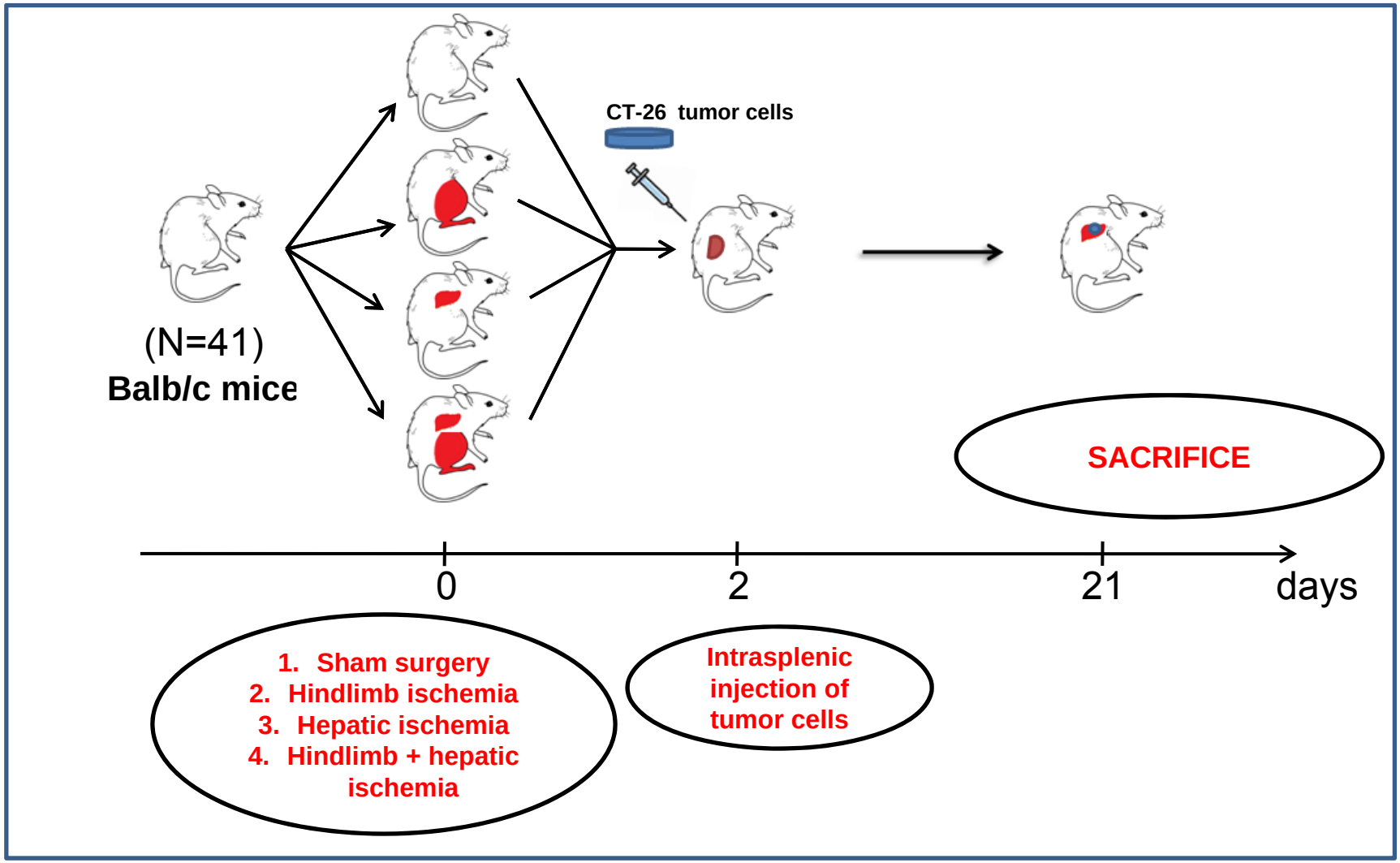
  (Ligation of the femoral artery)

- **Hepatic ischemia/reperfusion injury**

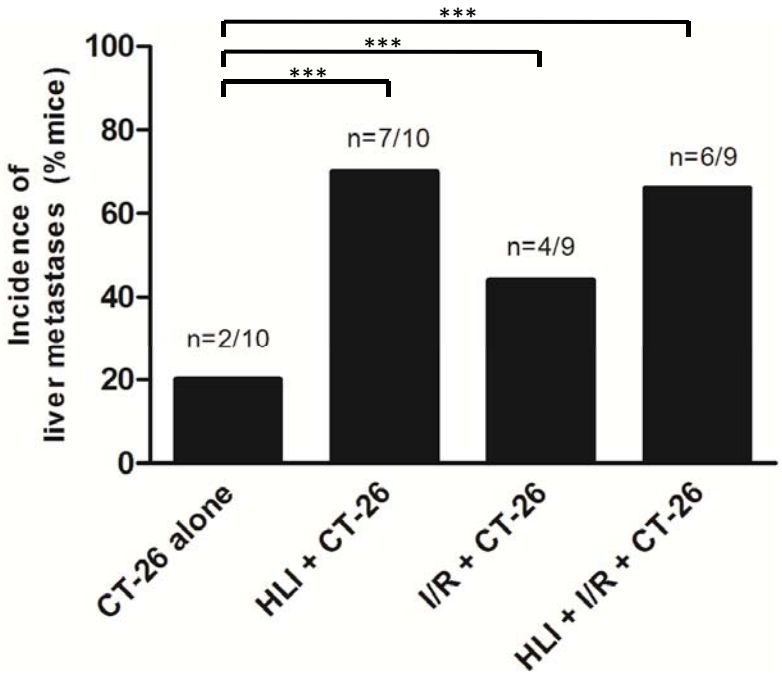
  (Hepatic pedicle clamping during 5 minutes)

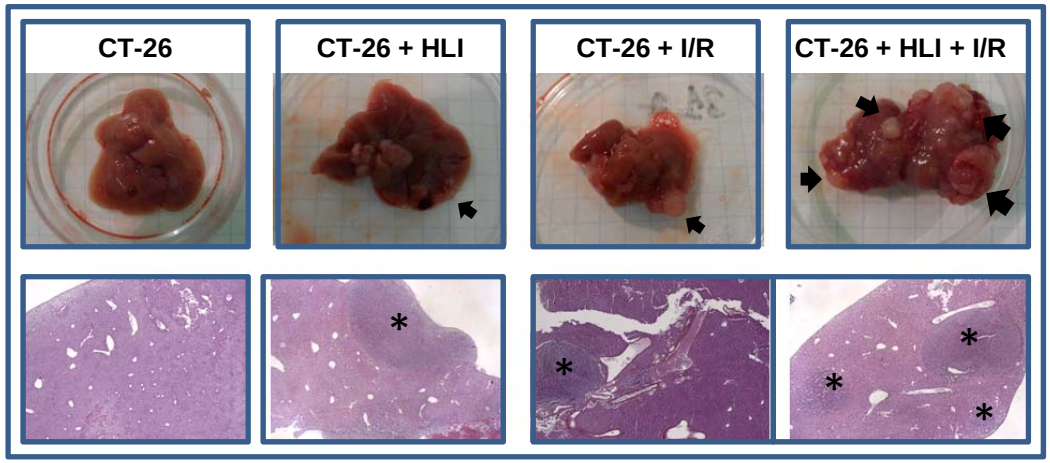


Experimental protocol



Ischemia increase incidence of liver metastasis

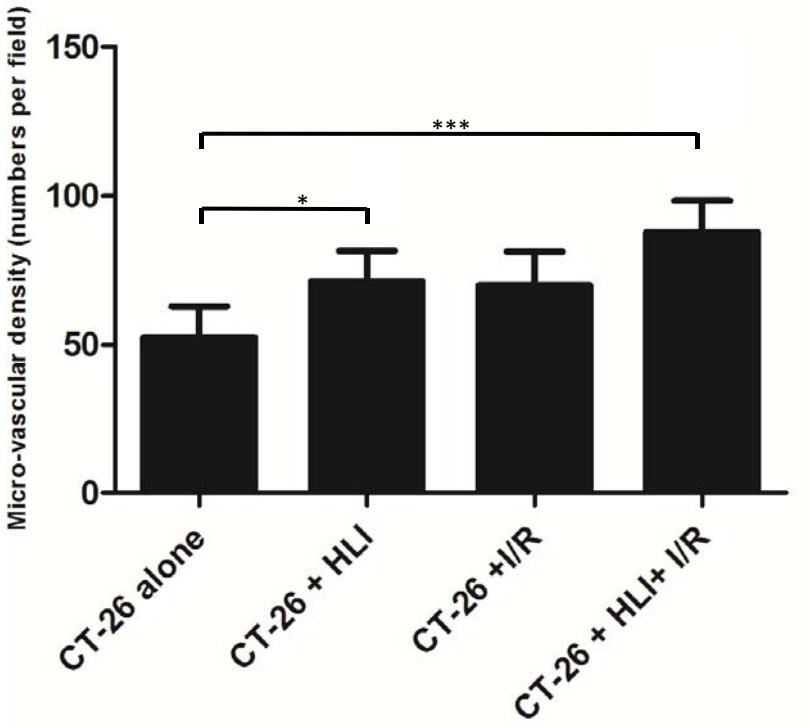




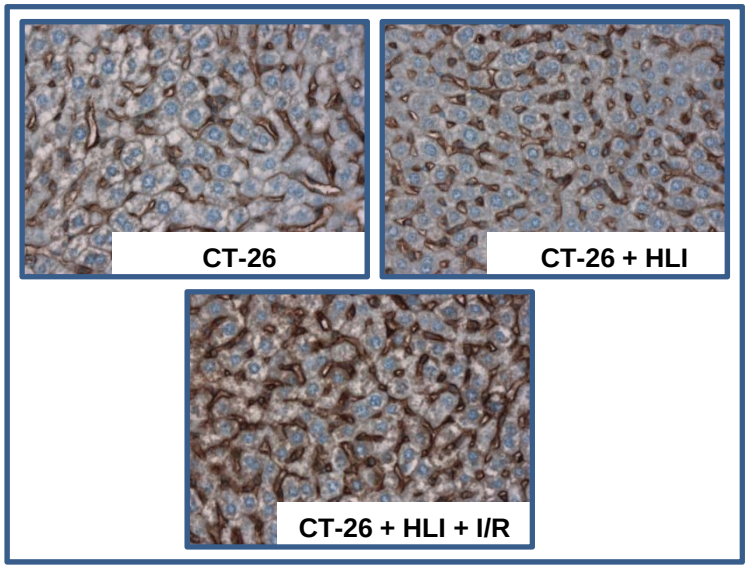
HLI: hind limb ischemia

I/R: ischemia/reperfusion

Ischemia increase non-tumorous liver parenchyma angiogenesis



Immunostaining with anti-CD31 antibody



Conclusion

- Local liver ischemia and distant ischemia induce:

  - ✓ a microenvironment modification
  - ✓ an increase of the metastatic potential
  - ✓ changes in angiogenesis in the surrounding liver parenchyma

JOURNAL OF SURGICAL RESEARCH 184 (2013) 888–897

Hepatic ischemia-reperfusion increases circulating bone marrow-derived progenitor cells and tumor growth in a mouse model of colorectal liver metastases

Chetana Lim, MD,[a,] Dong Broqueres-You, MD, PhD,[a,b,c] Jean-Philippe Brouland, MD, PhD,[a,d] Tatiana Merkulova-Rainon, MD,[a,b] Anne-Marie Faussat, MD,[e] Rose Hilal, MD,[a] Delphine Rouquie, MD,[a] Clarisse Eveno, MD,[a] Raphaëlle Audollent, MD,[a] Bernard I. Levy, MD, PhD,[a,b,c] and Marc Pocard, MD, PhD[a,f]*

N Engl J Med 2012;366:610-18

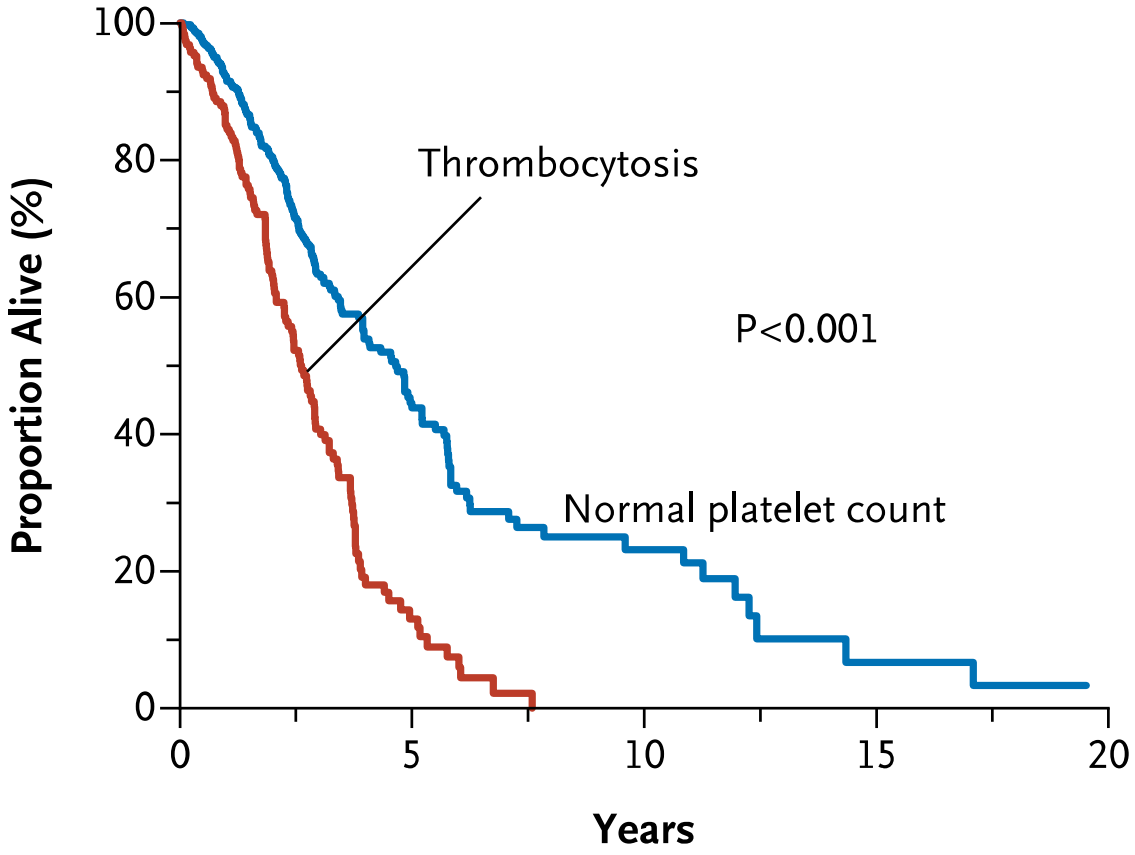
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Paraneoplastic Thrombocytosis in Ovarian Cancer

Rebecca L. Stone, M.D., Alpa M. Nick, M.D., Iain A. McNeish, Ph.D., Frances Balkwill, Ph.D., Hee Dong Han, Ph.D., Justin Bottsford-Miller, M.D., Rajesha Rupaimoole, M.S., Guillermo N. Armaiz-Pena, Ph.D., Chad V. Pecot, M.D., Jermaine Coward, Ph.D., Michael T. Deavers, M.D., Hernan G. Vasquez, Ph.D., Diana Urbauer, M.S., Charles N. Landen, M.D., Wei Hu, M.D., Ph.D., Hannah Gershenson, Koji Matsuo, M.D., Mian M.K. Shahzad, M.D., Erin R. King, M.D., Ibrahim Tekedereli, M.D., Bulent Ozpolat, M.D., Ph.D., Edward H. Ahn, M.D., Virginia K. Bond, M.D., Rui Wang, B.S., Angela F. Drew, Ph.D., Francisca Gushiken, M.D., Katherine Collins, B.S., Koen DeGeest, M.D., Susan K. Lutgendorf, Ph.D., Wah Chiu, Ph.D., Gabriel Lopez-Berestein, M.D., Vahid Afshar-Kharghan, M.D., and Anil K. Sood, M.D.

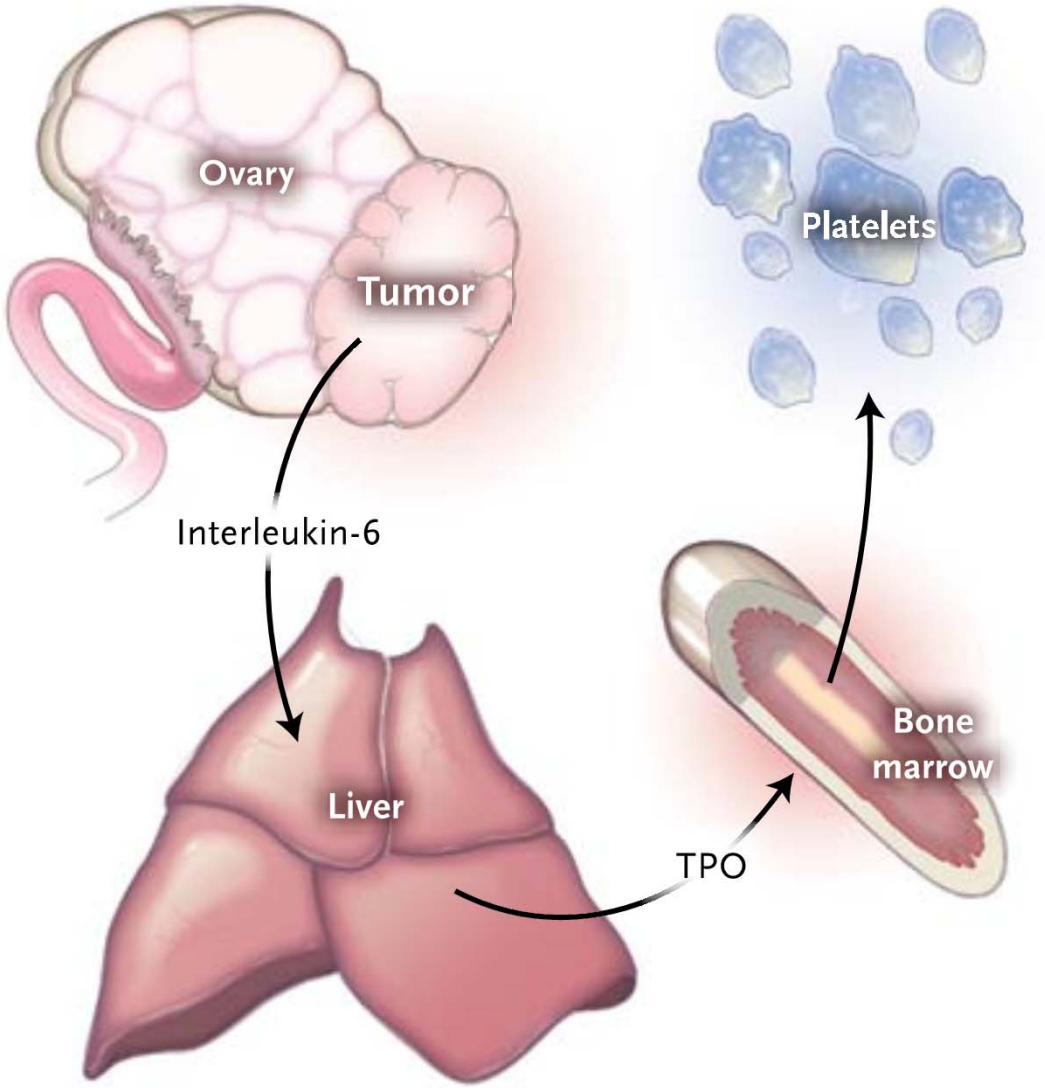
B **Overall Survival**



No. at Risk

Normal platelet count	428	191	57	21	13	3	2	1	0
Thrombocytosis	192	74	10	1	0	0	0	0	0

B **Proposed Paracrine Signaling Pathway**



ORIGINAL ARTICLE

Angiogenesis in Synchronous and Metachronous Colorectal Liver Metastases

The Liver as a Permissive Soil

Gesiena E. van der Wal, MSc, Annette S.H. Gouw, MD, PhD,† Jan A. A. M. Kamps, PhD,* Henk E. Moorlag, Ing,* Marian L. C. Bulthuis, Ing,† Grietje Molema, PhD,* and Koert P. de Jong, MD, PhD‡*

Ann Surg 2012;255:86–94

ORIGINAL ARTICLE

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The Liver as a Permissive Soil

Gesiena E. van der Wal, MSc, Annette S.H. Gouw, MD, PhD,† Jan A. A. M. Kamps, PhD,* Henk E. Moorlag, Ing,* Marian L. C. Bulthuis, Ing,† Grietje Molema, PhD,* and Koert P. de Jong, MD, PhD‡*

Patients

SS group: n=6; 4 solitary

Synchronous liver metastasis detected & removed sinmutaneously with primary CRC

LS group: n=10; 7 solitary

Synchronous liver metastasis removed 3-12 mois after primary CRC resection

M group: n=13; 11 solitary

Metachronous metastasis

Metastasis detected and resected later than 14 months after primary CRC resection

Methods

QPCR:

VEGF-A, PlGF, VEGFR-1, VEGFR-2

Ang-1, Ang-2, Tie-2

HIF-1α

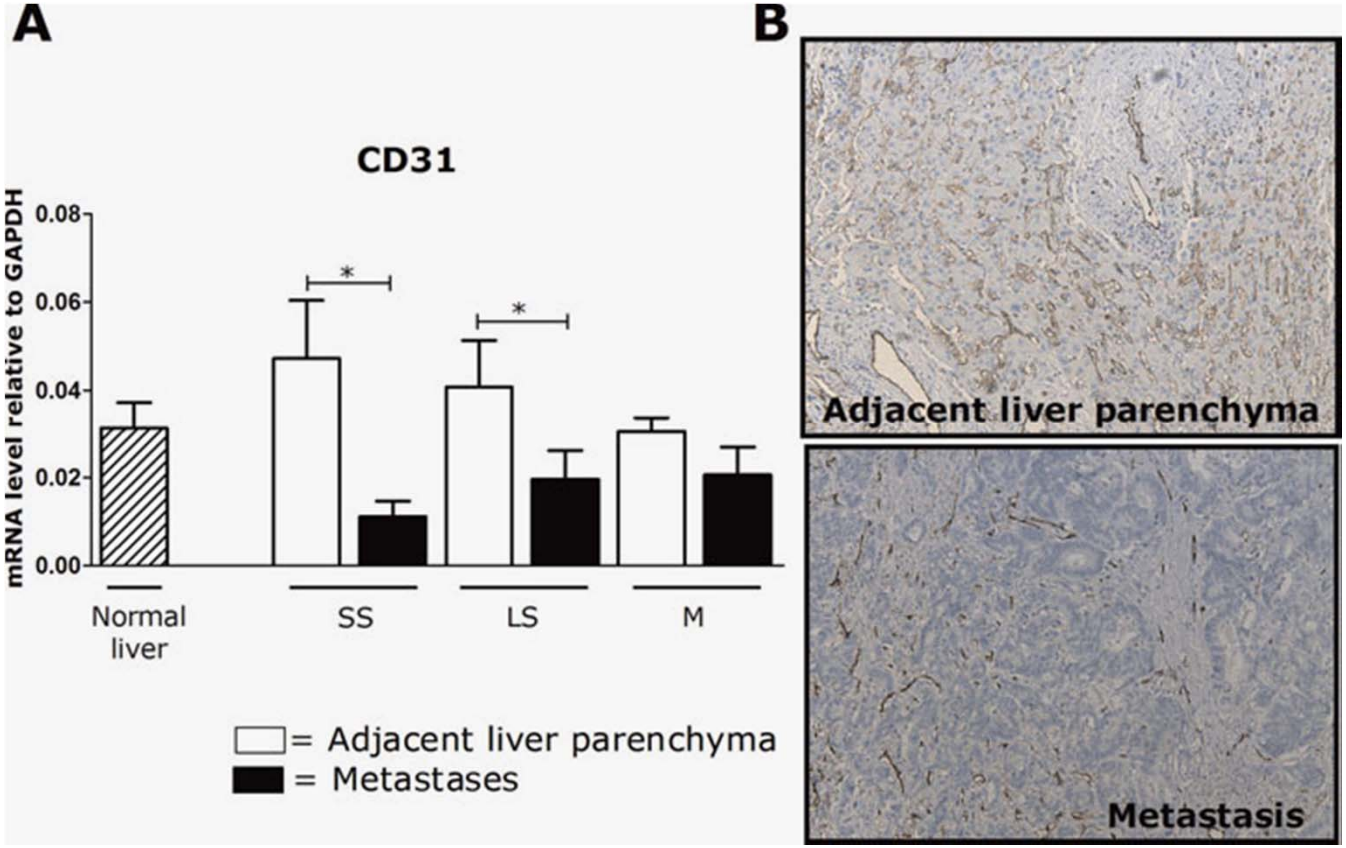
Proliferation/apoptosis

Ki67/caspase3a

Immunohistochemical stainings

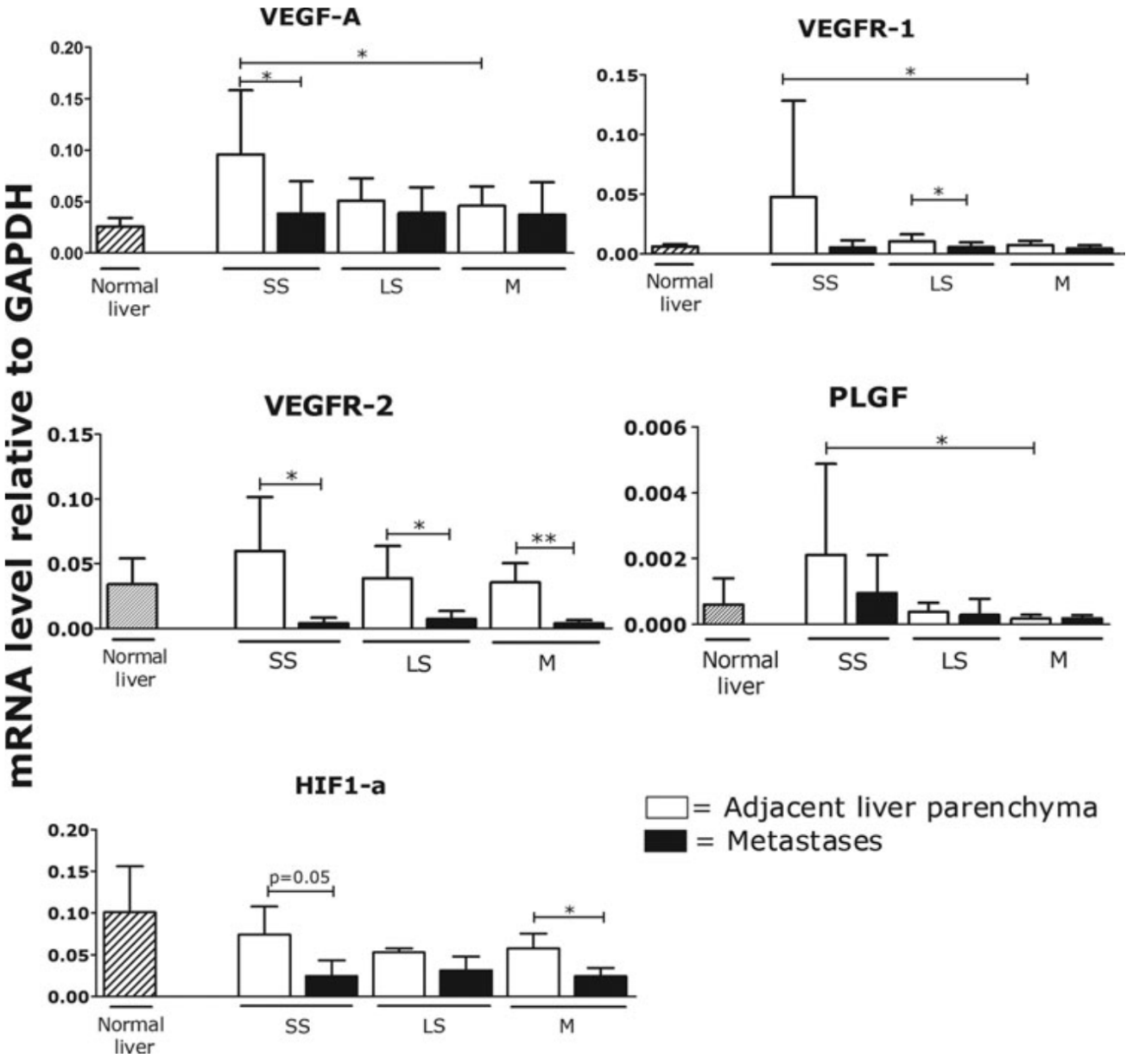
VEGF-A, VEGFR-2 & tie-2

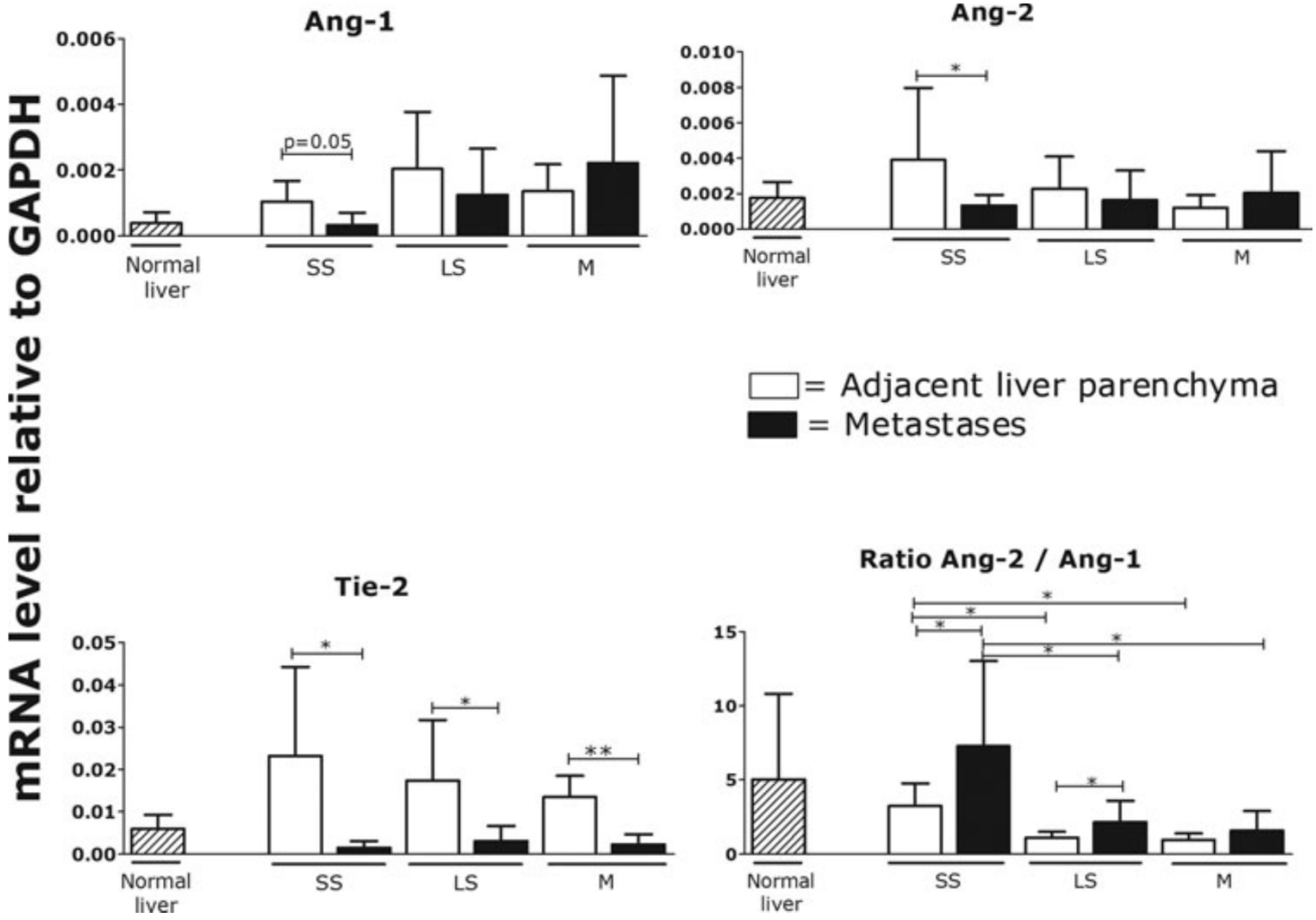
SMA/CD34



Taux de CD31mRNA: x 4 SS &
x2 LS groupes
Pas de différence M groupe

Méta moins vascularisée que tissu
Hépatique adjacent


VEGF-A
VEGFR-1
VEGFR-2
PLGF
HIF1-a
mRNA level relative to GAPDH
Normal liver
SS
LS
M
p=0.05
= Adjacent liver parenchyma
= Metastases


Ang-1
Ang-2
mRNA level relative to GAPDH
0.006
0.004
0.002
0.000
p=0.05
0.010
0.008
0.006
0.004
0.002
0.000
*
Normal liver
SS
LS
M
= Adjacent liver parenchyma
= Metastases
Tie-2
Ratio Ang-2 / Ang-1
0.05
0.04
0.03
0.02
0.01
0.00
15
10
5
0
*
**

Results: In all 3 groups a higher expression of the angiogenic factors was encountered in adjacent liver parenchyma as compared to the metastases

1. Adjacent Liver Parenchyma Exhibits the Most. Prosperous Angiogenic Environment in Simultaneously Resected Synchronous Liver Metastases: *VEGFR-2 gene expression was abundant in adjacent liver parenchyma in all 3 groups.VEGF-A and VEGFR-1 were prominent in adjacent parenchyma in the SS-group.*

2. Adjacent Liver Parenchyma and Metastases of the Simultaneous Synchronous Group Showed a Balance Toward Angiogenesis: *The SS-group showed the highest Ang-2/Ang-1 ratio both in the metastases and the adjacent liver*

3. Higher Apoptotic Rate and Lower Tumor Growth Rate in Simultaneously Resected Synchronous Liver Metastases ??

Final Conclusions and hypothesis

The main finding of this study is that in patients in whom the primary tumor is still present, liver parenchyma adjacent to the liver metastases provides a highly prosperous angiogenic environment

Concluding, in patients with colorectal liver metastases in whom the primary tumor is still present, adjacent liver parenchyma - the soil - behaves as an angiogenesis driven prosperous environment for metastatic growth.

Animal study for future treatment ?

OPEN ACCESS Freely available online

PLoS one

FTY720 Suppresses Liver Tumor Metastasis by Reducing the Population of Circulating Endothelial Progenitor Cells

Chang Xian Li, Yan Shao, Kevin T. P. Ng, Xiao Bing Liu, Chang Chun Ling, Yuen Yuen Ma, Wei Geng, Sheung Tat Fan, Chung Mau Lo, Kwan Man*

Department of Surgery, Centre for Cancer Research and State Key Laboratory of Liver Research,The University of Hong Kong, Hong Kong, China

La chirurgie colique de première intention est-elle indispensable chez le malade porteur de cancer colique avec métastases synchrones ?
Résultats d'une étude rétrospective multicentrique

I Sobhani, F Mesli, C Louvet, E Mitry, B Landi,
T Aparicio, et al.

- Gastroenterol Clin Biol 2009 ; 33 A 157 P.217
- 102 malades
- Différence de survie nette en faveur des patients ayant eu l'exérèse de la tumeur colique

Primary colectomy in patients with stage IV colon cancer and unresectable distant metastases improves overall survival: Results of a multicentric study

- M Karoui, F Roudot-Thoraval, F Mesli et al. **Dis colon Rectum** 2011 54:930-8.

- 4 facteurs liés à la survie de 208 patients avec colon stade 4 avec ECOG performance status > 1
- Résection curative secondaire : 46 mois versus 20,6
- Différentiation de la tumeur primitive: 10 mois versus 30
- Site métastatique hépatique isolé : 27 versus 18 (poumon)
- Présence d'un stent colique : 18 mois versus 25,7 (sans stent)

Une question ? Un dogme ?

En cas de cancer métastatique synchrone faut-il réaliser l'exérèse de la tumeur primitive ?

Question complexe

Probablement une question générique pour toute la cancérologie solide.

Surgical resection of the primary tumour is associated with improved survival in patients with distant metastatic breast cancer at diagnosis.

J Ruiterkamp, MF Ernst, LV van de Poll-Franse et al.
E J S O 2009;35:1146-51

728 patientes avec des métastases a distance

Survie médiane : 31 mois versus 14 et

Survie à 5 ans : 24% versus 13% ($p<0.0001$)

Nephrectomy followed by interferon alfa-2b compared with interferon alfa-2b alone for metastatic renal cell-cancer

RC Flanigan, SE Salmon, BA Blumenstein et al.
N Engl J Med 2001;345:1655-9.

241 patients (âge moyen 60 ans)

Survie avec néphrectomie 11 mois versus 8 mois (p=0.04)

Radical nephrectomy plus interferon alfa-based immunotherapy compared with interferon alfa alone in metastatic renal-cell cancer

GH Mickisch, A Garin, H van Poppel, et al (EORTC).
Lancet 2001;358:966-70.

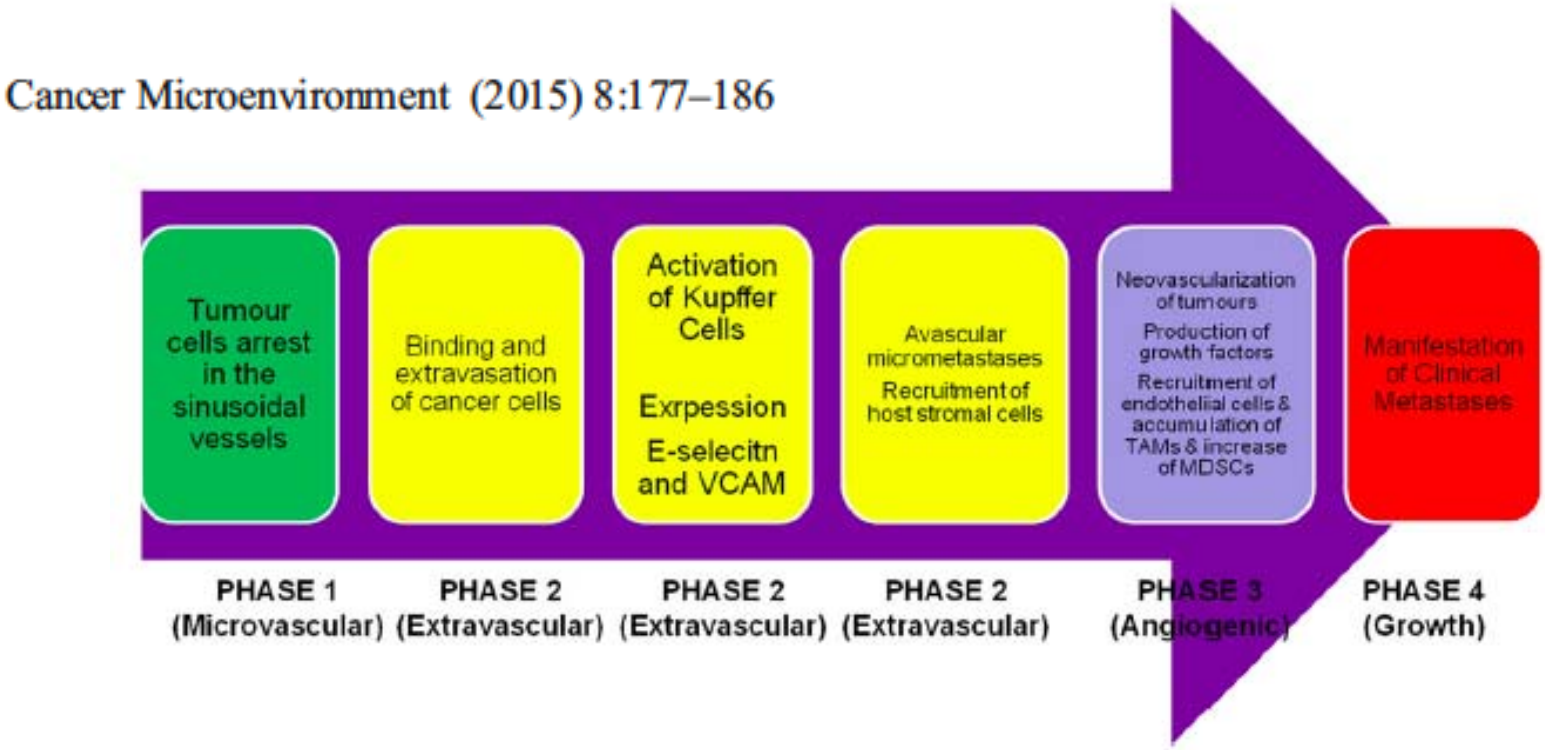
84 patients

Survie avec néphrectomie 17 mois versus 7 mois (p=0.03)

En conclusion

- Le processus métastatique est bien plus complexe que prévu
- Le microenvironnement est modulé par la tumeur primitive via l'angiogenèse et les promoteurs dérivés de la moelle
- L'ablation de la tumeur primitive pourrait diminuer le caractère permissif du foie pour le processus métastatique
- Les traitements associés à la chirurgie de demain devront cibler ce microenvironnement et non plus uniquement les cellules tumorales.

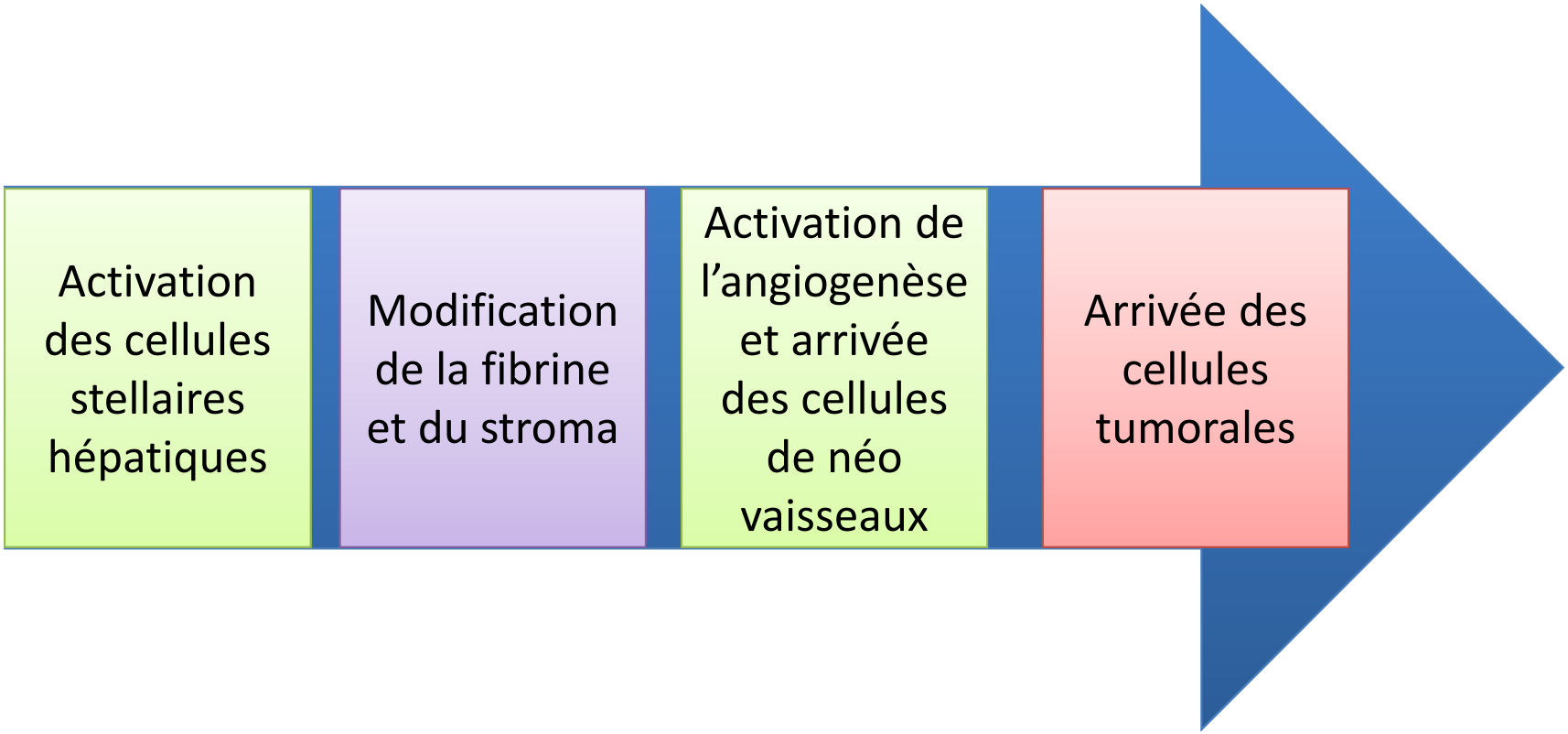
Ancienne conception du processus métastatique hépatique



- Les cellules tumorales arrivent en premier – s'installent par des phénomènes physiques puis après une croissance initiale activent l'angiogenèse

Tumour Microenvironment: Overview with an Emphasis on the Colorectal Liver Metastasis Pathway

Nouvelle conception du processus métastatique hépatique



- Les cellules tumorales arrivent après que ce soit produit des modifications rendant efficace la niche métastatique

Donc ce qui change

- Inutile de déterminer des facteurs pronostics des cellules tumorales coliques puisque le phénomène implique d'autres structures et d'autres type cellulaire
- Signature failure

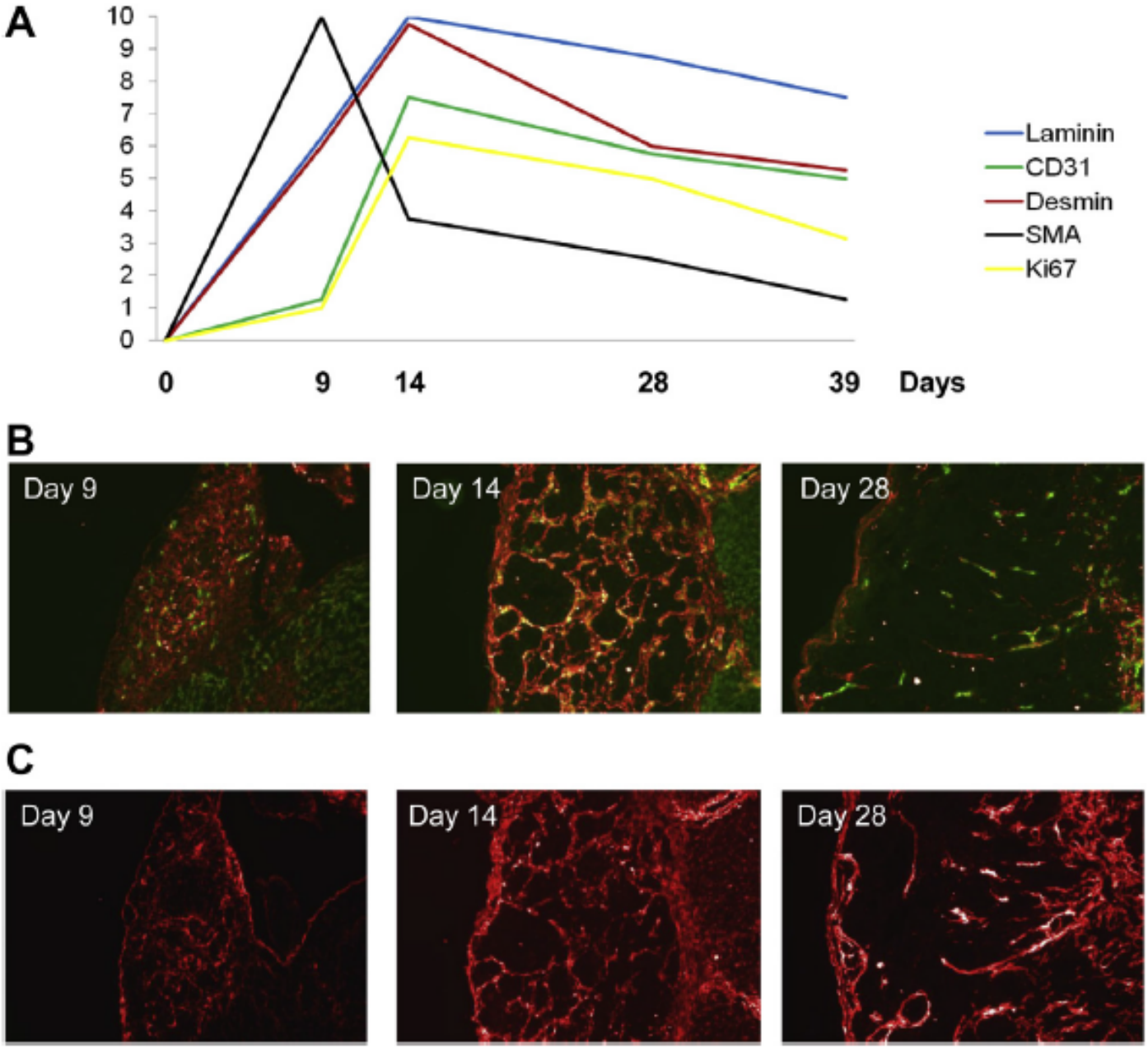
- Cibler la cellule tumorale est un combat d'arrière garde puisque les conditions sont déjà remplies pour permettre à celles qui restent de croitre

- Cibler le microenvironnement semble plus logique ?

JOURNAL OF SURGICAL RESEARCH 194 (2015) 496–504

Proof of prometastatic niche induction by hepatic stellate cells

Clarisse Eveno, PhD, MD,[a,b] *Patricia Hainaud, PhD,*[a]
Aurore Rampanou, BA,[a] *Philippe Bonnin, PhD, MD,*[a,c]
Sana Bakhouche, BA,[a] *Evelyne Dupuy, PhD, MD,*[a]
Jean-Olivier Contreres, PhD,[a,1] *and Marc Pocard, PhD, MD*[a,b,*]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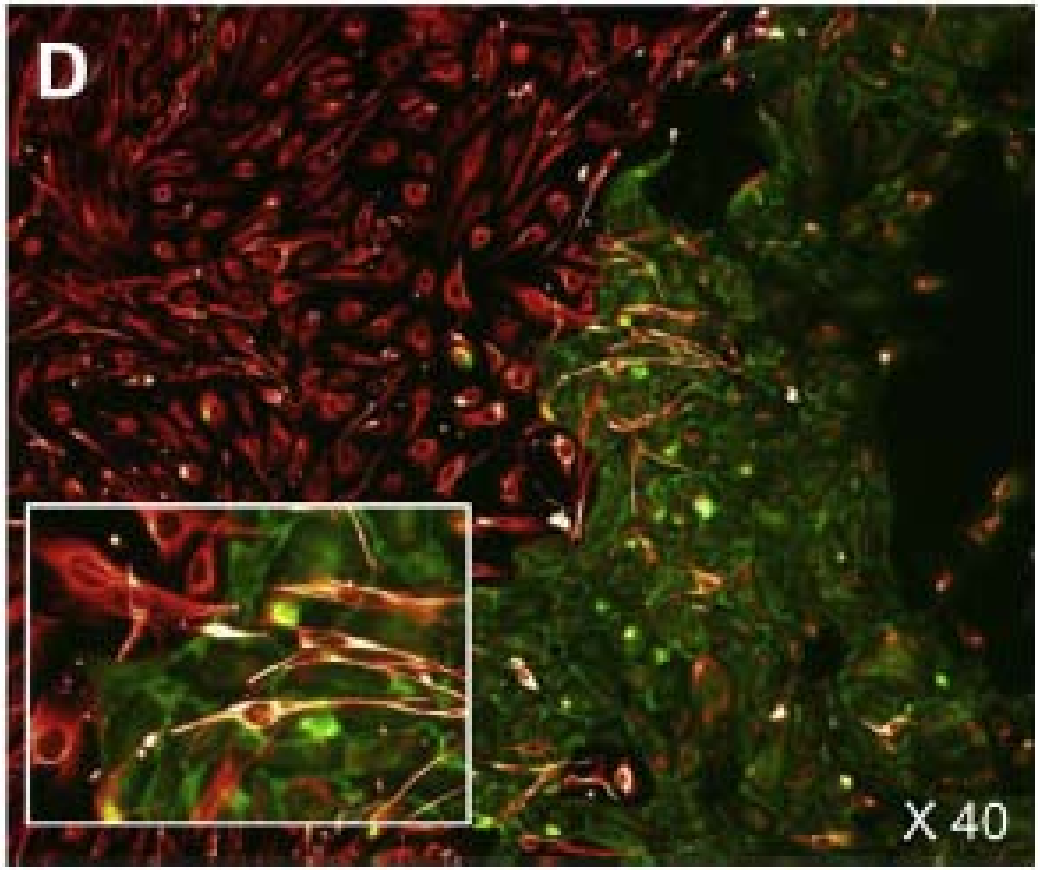
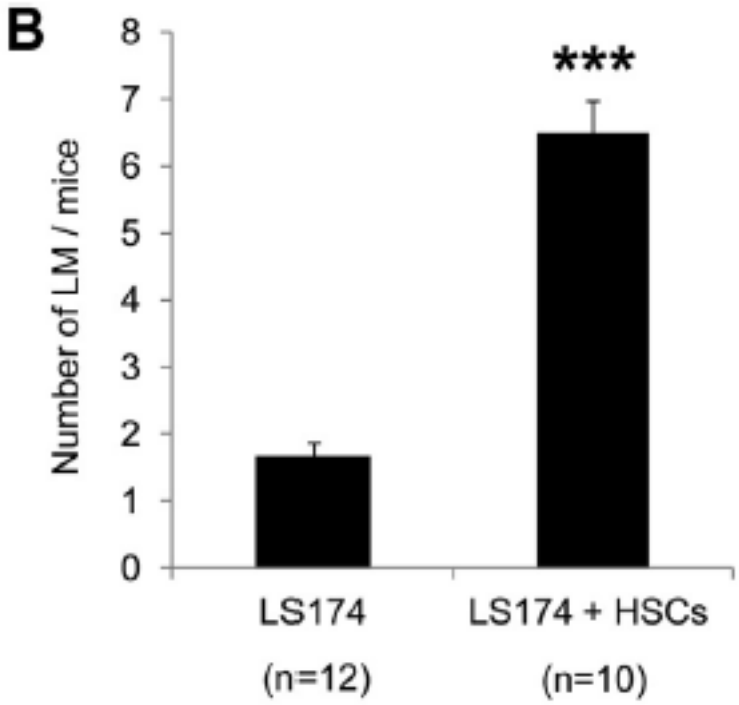




When cocultured with HUVECs, HSCs interact through the extension of long filopods. Original magnification ×20; inset ×100.

Clinical Application of Basic Science

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Stellate cells and the development of liver cancer: Therapeutic potential of targeting the stroma

Cédric Coulouarn*, Bruno Clément

Inserm, UMR991, Liver Metabolisms and Cancer, F-35033 Rennes, France; Université de Rennes 1, F-35043 Rennes, France

Journal of Hepatology **2014** vol. 60 | 1306–1309

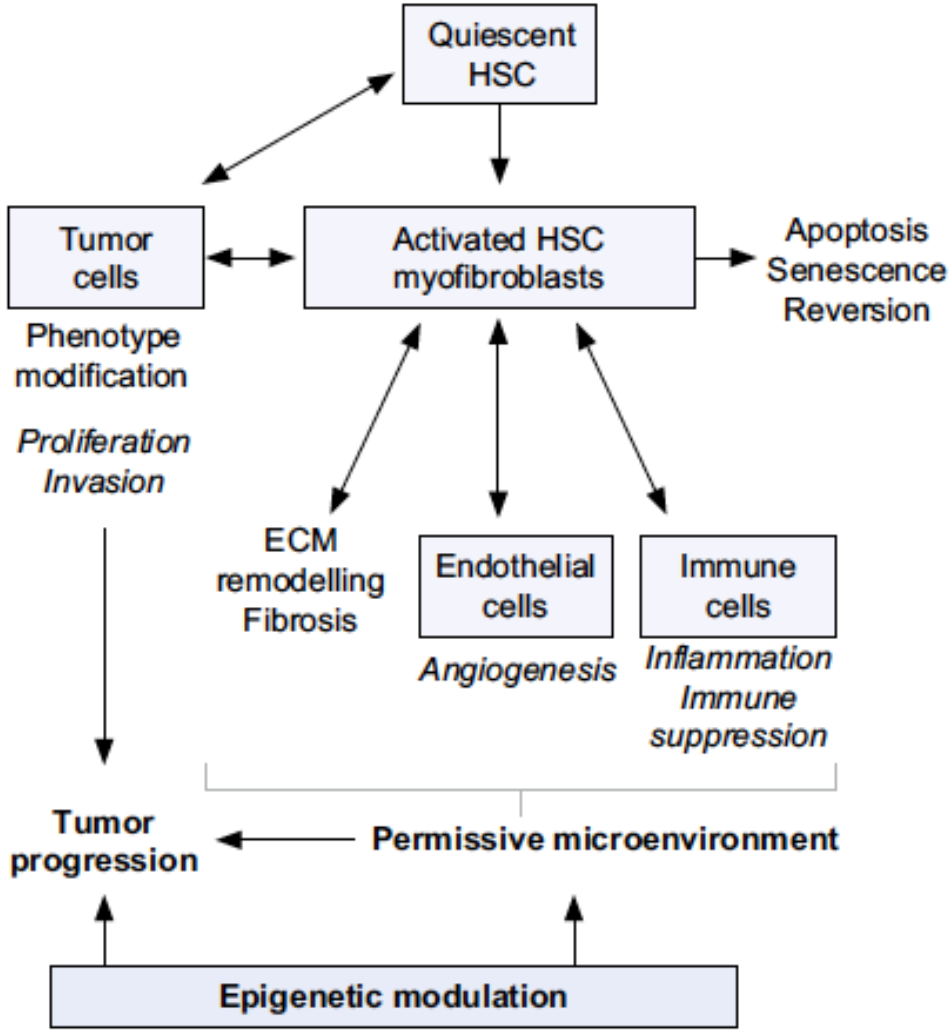
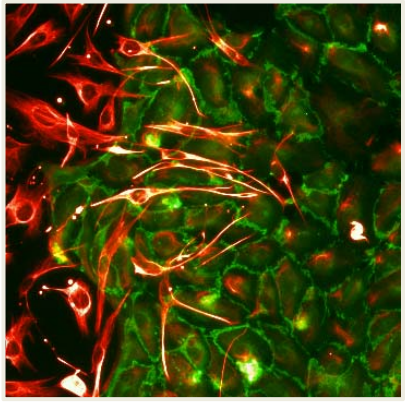
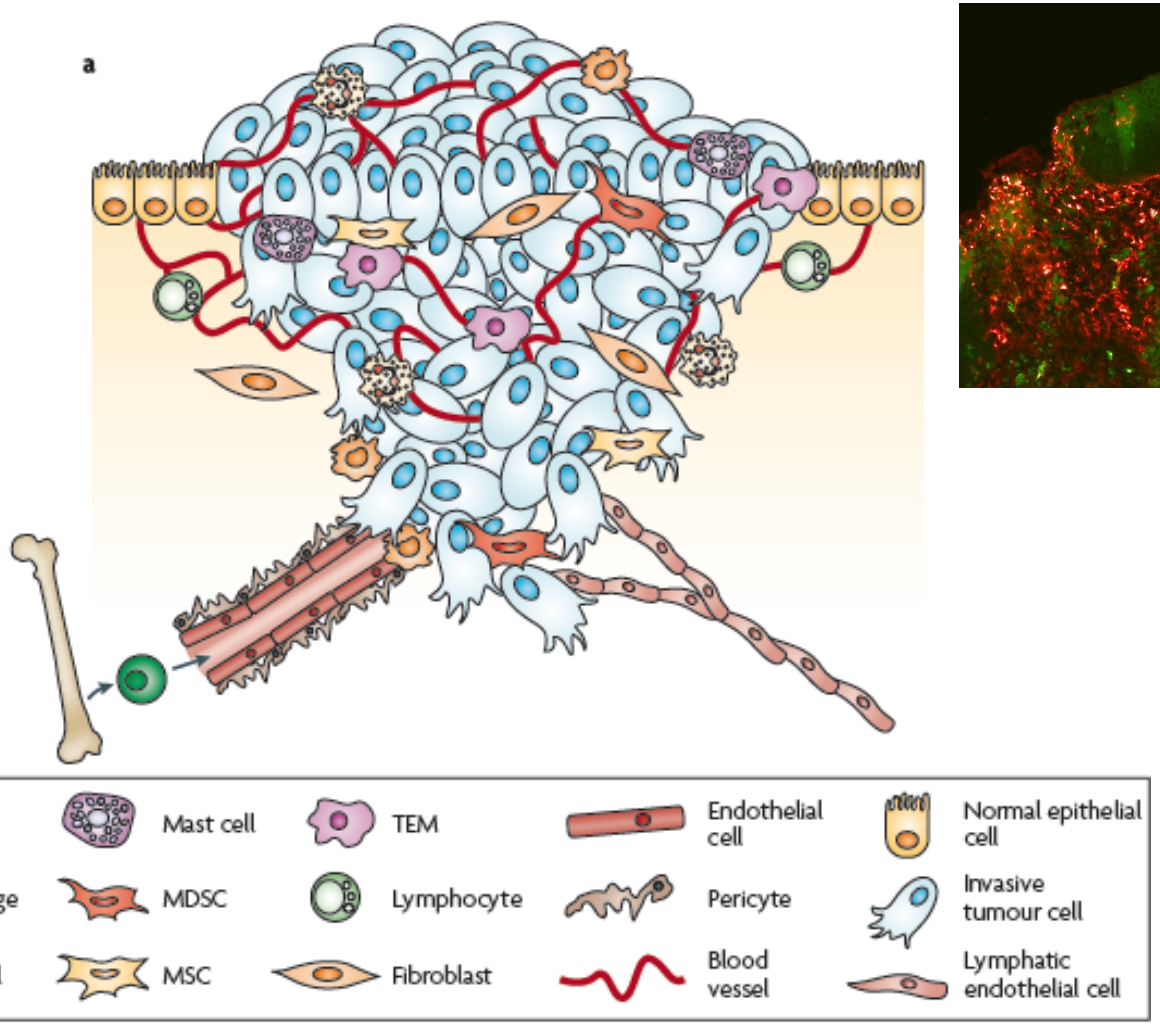
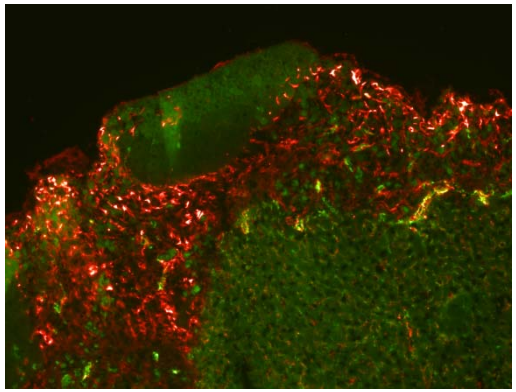


Fig. 1. Therapeutic potential of targeting HSC and the stroma in liver cancer. Depicted is the interplay between tumors cells and components of the microenvironment, notably HSC, which influence tumor progression by creating a permissive environment and by perverting the phenotype of tumor cells. Each of these interactions, symbolized by arrows, could be targeted for therapeutic purpose. Epigenetic modulation of the crosstalk between tumor cells and their microenvironment may also open new therapeutic opportunities in liver cancers.

The tumor microenvironment







Joyce and Pollard. Nature Review Cancer. 2009

Que cibler après la chirurgie, pour aider Serge Evrard ?

- Le système immunitaire
- Les cellules stellaires
- Les cytokine qui induisent la niche : VEGFR2
- En adjuvant ceux qui ont échoués en métastatique : anti IGFR1 – MMP9

Microenvironment and Immunology

Cancer Research

Insulin-Like Growth Factor-I Regulates the Liver Microenvironment in Obese Mice and Promotes Liver Metastasis

Yingjie Wu[1], Pnina Brodt[2], Hui Sun[1], Wilson Mejia[1], Ruslan Novosyadlyy[1], Nomeli Nunez[3], Xiaoli Chen[4], Arnulfo Mendoza[5], Sung-Hyeok Hong[5], Chand Khanna[5], and Shoshana Yakar[1]

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Original Articles

Hepatic stellate cells activated by acidic tumor microenvironment promote the metastasis of hepatocellular carcinoma via osteopontin

Jin Song [a,1], Zhouhong Ge [a,1], Xinrong Yang [b,1], Qin Luo [a], Cun Wang [a], Haiyan You [a], Tianxiang Ge [a], Yun Deng [a], Hechun Lin [a], Yongqi Cui [a], Wei Chu [a], Ming Yao [a], Zhigang Zhang [a], Jianren Gu [a], Jia Fan [b,*], Wenxin Qin [a,**]

Ann Surg Oncol (2015) 22:S1516–S1523
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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

KISS1 Associates with Better Outcome via Inhibiting Matrix Metalloproteinase-9 in Colorectal Liver Metastasis

Chengzhan Zhu, MD[1,2], Chie Takasu, MD, PhD[1], Yuji Morine, MD, PhD[1], Yoshimi Bando, MD, PhD[3], Tetsuya Ikemoto, MD, PhD[1], Yu Saito, MD, PhD[1,2], Shinichiro Yamada, MD[1,2], Satoru Imura, MD, PhD[1], Yusuke Arakawa, MD, PhD[1], and Mitsuo Shimada, MD, PhD[1]

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